

## Volatile Constituents of Whey Powder Subjected to Accelerated Browning

### Abstract

The volatile constituents of whey powder subjected to accelerated browning were isolated and identified. Fifty-five compounds were identified by gas-liquid chromatography and mass spectrometry. Of these, the following have not been reported in milk-related systems: benzofuran, furfuryl propionate, 5-methyl-2-furfuryl alcohol, furfuryl butyrate, 1-(2'-furyl)-propane-1,2-dione, 2-furyl hydroxymethyl ketone, 2-methyl-5-furfuryl 2'-furfuryl ether, 5-(5'-methyl-2'-furfuryl)-2-furaldehyde, 5-methyl-2-formylpyrrole, *N*-ethyl-2-formylpyrrole, 4-methyl-3-butenic acid- $\gamma$ -lactone, and hexane-2,5-dione. Flavor and potency of 5-(2'-furfuryl)-2-furaldehyde and of 5-(5'-methyl-2'-furfuryl)-2-furaldehyde suggests that not only lipids but also carbohydrates must be considered as possible precursors of the oxidized flavor in dairy products.

### Introduction

The Maillard reaction is important in staling and general deterioration of milk products (1, 12). Indeed it has long been demonstrated that, during processing and storage, changes occur involving the carbohydrate and protein constituents (1, 6). Whey is considered more prone to browning-type deterioration than non-fat dry milk (1). Our work is part of a long time program (2, 3) designed to assess the importance of various milk fractions in the nonenzymatic browning reaction in skim milk powders, in relation to off-flavor development. We report the identification of volatile constituents in freeze-dried whey powder subjected to accelerated browning.

### Experimental Procedures<sup>1</sup>

*Preparation of whey powder.* Fresh pasteurized skim milk from cows of the Beltsville herd

was centrifuged at  $34,800 \times g$  at 25 C for 30 min with a Sorvall Superspeed RC-2 automatic refrigerated centrifuge. The greenish-yellow clear portion of the supernatant was pipetted from the tube, avoiding the small amount of milk fat at the top, concentrated to one-third its original volume with a rotary evaporator at 10 to 15 C under reduced pressure, and finally freeze-dried with a Virtis freeze-mobile apparatus. From 12.3 liters of skim milk we obtained 8.7 liters of whey which, after concentration, yielded 639 g of freeze-dried powder.

*Accelerated browning of whey.* Five hundred grams of freeze-dried whey powder were subjected to accelerated browning at 70 C in an atmosphere of 75% relative humidity for 4 days. The browned mass was extracted in a Soxhlet apparatus for 20 hr with dichloromethane. The solvent was carefully evaporated, and the residue was distilled under high vacuum (0.1 mm) allowing the volatile compounds to condense on a cold finger placed 5 cm from the surface of the material being distilled, as previously reported (3). Initial and final bath temperatures were -20 and 100 C. The resulting 1.1 g of distillate will be referred to as "heated whey extract" which was analyzed.

*Gas-liquid chromatography (GLC) and mass spectrometry (MS).* Chromatographic separations were performed with stainless steel columns with a glass liner at the injection port under the following conditions. Column A: 3-m  $\times$  6.35-mm (od) packed with 15% Triton X305 plus 0.15% Versamid 900, on 60/80 Chromosorb W (AW/DMCS treated). Temperature programming was at 4 degrees per minute from 70 to 200 C and isothermal thereafter. Column B: 3-m  $\times$  6.35-mm (od) packed with 20% Silicone SE-30 on 60/80 Chromosorb W (AW/DMCS treated). The column was programmed at 2 degrees per minute from 75 to 200 C and isothermal thereafter. Column C: 2.5-m  $\times$  3.17-mm (od) packed with 10% Silicone SE-30 on 100/120 Chromosorb W (AW/DMCS treated). Temperature programming was varied, depending on the fraction being analyzed.

The instrument was a 5750 Hewlett-Packard equipped with a thermal conductivity cell maintained at 235 C. For mass spectral analysis,

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<sup>1</sup> Mention of brand or firm names does not constitute an endorsement by the Department of Agriculture over others of a similar nature not mentioned.

TABLE 1. Compounds identified in whey powder subjected to accelerated browning. (Continued)

Compound	Mass spectra, m/e
2-Furaldehyde <sup>a</sup>	
Furfuryl formate <sup>a</sup>	
2-Acetylfuran <sup>a</sup>	
Furfuryl methyl ketone <sup>a</sup>	
5-Methyl-2-furaldehyde <sup>a</sup>	
Furfuryl acetate <sup>a</sup>	
Benzofuran	118M <sup>+</sup> (100), 90(40), 89(37), 63(21), 62(9), 39(9), 119(8), 64(6)
Furfuryl alcohol <sup>a</sup>	
2-Propionylfuran <sup>a</sup>	
5-Methyl-2-acetylfuran <sup>a</sup>	
2, 2'-Bifuran <sup>a</sup>	
Furfuryl propionate	81(100), 98(41), 57(30), 154M <sup>+</sup> (29), 52(28), 53(26), 29(26), 27(20)
2, 2'-Difurylmethane <sup>a</sup>	
2-Methylbenzofuran <sup>a</sup>	
5-Methyl-2-furfuryl alcohol	95(100), 43(76), 112M <sup>+</sup> (61), 41(38), 97(30), 111(27), 55(26), 39(26)
Difurfuryl ether <sup>a</sup>	
[2-Furyl-(2'-methyl-5'-furyl)] methane <sup>a</sup>	
Furfuryl butyrate	81(100), 98(34), 43(31), 27(24), 52(23), 53(22), 71(19), 168M <sup>+</sup> (15)
1-(2'-Furyl)-propane-1, 2-dione	95(100), 43(44), 39(26), 138M <sup>+</sup> (15), 38(8), 96(6), 15(6), 67(5)
2-Furyl hydroxymethyl ketone <sup>b</sup>	95(> 100), 39(100), 126M <sup>+</sup> (50), 96(28), 31(22), 67(20), 51(10), 41(8)
5-(2'-Furfuryl)-2-furaldehyde <sup>a</sup>	
2-Methyl-5-furfuryl 2'-furfuryl ether <sup>c</sup>	39(11), 27(7), 52(7)
2-Methyl-5-(5'-methyl-2'-furfuryl) furan <sup>a</sup>	81(100), 95(42), 192M <sup>+</sup> (24), 53(22), 96(13), 190M <sup>+</sup> (100), 43(51), 161(21), 91(19), 175(17), 105(16), 53(15), 51(15)
5-(5'-Methyl-2'-furfuryl)-2-furaldehyde	
5-Hydroxymethyl-2-furaldehyde <sup>a</sup>	
2-[2'-Furfuryl]-5-[2''-furfuryl] furan <sup>a</sup>	
n-Methyl-2-formylpyrrole <sup>a</sup>	
5-Methyl-2-formylpyrrole	109M <sup>+</sup> (100), 108(87), 80(47), 53(42), 52(13), 51(12), 27(12), 39(7)
n-Ethyl-2-formylpyrrole	123M <sup>+</sup> (100), 94(60), 122(35), 108(30), 106(19), 39(18), 80(13), 66(13)
2-Acetylpyrrole <sup>a</sup>	
γ-Butyrolactone <sup>a</sup>	
β-Hydroxy-γ-butyrolactone <sup>a</sup>	
β-Acetyl-γ-butyrolactone <sup>a, c</sup>	
4, 5-Dihydroxyvaleric acid-γ-lactone <sup>a</sup>	
2-Butenoic acid-γ-lactone <sup>a</sup>	
3-Butenoic acid-γ-lactone <sup>a</sup>	
4-Methyl-2-butenic acid-γ-lactone <sup>a</sup>	
4-Methyl-3-butenic acid-γ-lactone	55(100), 43(87), 98M <sup>+</sup> (64), 27(44), 26(16), 42(15), 70(10), 39(14)
Protoanemonin <sup>a</sup>	
Acetamide <sup>a</sup>	
n-Methyl-2-pyrrolidinone <sup>a</sup>	
Glutarimide <sup>a</sup>	
Acetol <sup>a</sup>	
3-Hydroxy-2-butanone <sup>a</sup>	

TABLE 1. Compounds identified in whey powder subjected to accelerated browning. (Concluded)

Compound	Mass spectra, m/e
Hexane-2, 5-dione	43(100), 99(28), 71(15), 15(8), 57(6), 27(6), 114M <sup>+</sup> (5), 42(5)
2-Methyl-tetrahydrofuran-3-one <sup>a</sup>	
Benzaldehyde <sup>a</sup>	
Phenol <sup>a</sup>	
<i>p</i> -Cresol	107(100), 108M <sup>+</sup> (86), 77(22), 79(17), 51(13), 39(11), 53(10), 78(10)
Maltol <sup>a</sup>	
Dimethylsulfone	79(100), 15(59), 94M <sup>+</sup> (51), 49(9), 45(8), 48(6), 29(6), 63(5)
Acetic acid <sup>a</sup>	
Propionic acid <sup>a</sup>	
Octanoic acid	60(100), 73(69), 43(46), 41(33), 55(28), 85(21), 29(20), 27(18)
Acetol acetate <sup>a</sup>	

<sup>a</sup> Compound also identified by the authors in a lactose-casein model system (References 2, 3).

<sup>b</sup> Dehydrogenation of the alcoholic group occurred during the mass spectrum determination.

<sup>c</sup> Tentative identification (see Results).

components leaving the end of Column C from the instrument were introduced into the separator of an LKB-9000 mas spectrometer with a silanized 1.6 mm (od) stainless steel tube heated at about 200 C. Other mass spectral conditions were: ionizing energy 70 ev, ion source pressure  $5 \times 10^{-6}$  mm; ion source temperature 290 C; acceleration voltage 3.5 kv; electron multiplier voltage 2.5 kv; exit slit 0.3 mm; separator at 235 C.

In all cases the carrier gas (He) was supplied at 2.8 kg/cm<sup>2</sup>, and the injection block was 235 C.

*GLC-MS analysis of heated whey extract.* The dichloromethane extract of heated whey was fractionated into 18 cuts with Column A. Each cut was then resolved into its components on Column C and the effluent scanned with the mass spectrometer according to the usual procedure (2, 3). The high phase Column B (3) separated and removed, from certain cuts, relatively large quantities of furfuryl alcohol, 2-butenic acid- $\gamma$ -lactone,  $\gamma$ -butyrolactone and dimethyl sulfone.

*Reference compounds.* Authentic flavor components which were also found previously in a lactose-casein model system (Table 1) were obtained or synthesized as reported earlier (2, 3). Furfuryl propionate (14), furfuryl butyrate (14), 1-(2'-furyl)-propane-1, 2-dione (5), 2-furyl hydroxymethyl ketone (11), and 5-methyl-2-furfuryl alcohol (10) were prepared by published methods.

4-Methyl-3-butenic acid- $\gamma$ -lactone was obtained, along with the  $\alpha$ -unsaturated isomer, by slow distillation of levulinic acid.

5-(5'-Methyl-2'-furfuryl)-2-furaldehyde was prepared by the Vilsmeier-Haack formylation of [2-furyl-(2'-methyl-5'-furyl)] methane (2) following the same procedure used for the synthesis of 5-(2'-furfuryl)-2-furaldehyde (3), except that the crude product was prepurified by steam distillation rather than by chromatography over neutral alumina. This aldehyde has not been reported in the literature, and the infrared data of the GLC-purified material are given. IR(CCl<sub>4</sub> solution with a Beckman IR-5A instrument, beam condenser, and 0.1 mm cell); 3125w, 2933m, 2817s, 1681vs, 1618m, 1570s, 1508s, 1450m, 1418m, 1401s, 1312m, 1279m, 1244m, 1220s, 1200s, 1156w, 1020vs, 998m, 980s, 970s, 951m, 883w.

N-Ethyl-2-formylpyrrole was synthesized by the Vilsmeier-Haack formylation of N-ethylpyrrole which was obtained by reaction of 2,5-dimethoxytetrahydrofuran with 50% aqueous ethylamine in glacial acetic acid.

5-Methyl-2-formylpyrrole was obtained by the Wolff-Kishner reduction of 2-formylpyrrole followed by formylation (usual method) of the resulting 2-methylpyrrole.

The synthesis of 2-methyl-5-furfuryl 2'-furfuryl ether by acid catalyzed condensation of furfuryl alcohol with 5-methyl-2-furfuryl alcohol was unsuccessful; a great number of products resulted from which the desired ether could not be isolated.

All other compounds were from reliable commercial sources. All reference materials were purified by GLC.

### Results

The volatile compounds identified in the heated whey extract are in Table 1. Compounds were considered positively identified if their mass spectra matched spectra of authentic materials determined with our instrument, and their retention times on Columns A and C agreed with those of the reference compounds. All products were positively identified except 2-methyl-5-furfuryl 2'-furfuryl ether, an authentic sample of which could not be obtained, and the previously reported  $\beta$ -acetyl- $\gamma$ -butyrolactone (2). Table 1 also shows the relative intensities (in parentheses) of the eight most intense peaks (m/e) for the compounds identified in the present work but which were not found in the lactose-casein model system (2, 3). M<sup>+</sup> designates the molecular ion.

### Discussion

The data in Table 1 reveal that the nonenzymatic browning of whey powder under accelerated conditions produces many of the products which were observed in the lactose-casein model system. Namely, of the 55 compounds in Table 1, 40 were identified also in the latter system (2, 3). For discussion of their origin or mechanism of formation the reader is referred to previous publications by the authors (2, 3). Of the remaining ones the following have not been reported in dairy products: benzofuran, furfuryl propionate, 5-methyl-2-furfuryl alcohol, furfuryl butyrate, 1-(2'-furyl)-propane-1, 2-dione, 2-furyl hydroxymethyl ketone, 2-methyl-5-furfuryl 2'-furfuryl ether, 5-(5'-methyl-2'-furfuryl)-2-furaldehyde, 5-methyl-2-formylpyrrole, N-ethyl-2-formylpyrrole, 4-methyl-3-butenic acid- $\gamma$ -lactone, and hexane-2,5-dione. Their formation can be rationalized in accepted pathways of the Maillard reaction or sugar caramelization (7, 8). In particular, 5-methyl-2-furfuryl alcohol, hexane-2,5-dione, and even propionic acid, form by base-catalyzed degradation of hexoses (9, 13). Furfuryl propionate and furfuryl butyrate must have formed by esterification of furfuryl alcohol. Consequently, furfuryl butyrate is a product of mixed carbohydrate-lipid origin.

The most abundant volatile constituents of the heated whey extract were maltol, 2-acetylfuran, furfuryl alcohol, acetic acid, and dimethylsulfone. The latter is a well-known constituent of fresh milk, and was first identified in butteroil by Forss et al. (4).  $\gamma$ -Butyro-

lactone, which was first considered of lipid origin, has recently been obtained by base-catalyzed degradation of fructose (13), and in our system it probably formed by degradation of lactose. Octanoic acid no doubt originated from traces of residual fat in whey. Unexpected is the absence, in heated whey extract, of alkylpyrazines which were found in the lactose-casein system heated under similar conditions (2, 3).

5-(2'-Furfuryl)-2-furaldehyde and 5-(5'-methyl-2'-furfuryl)-2-furaldehyde are believed to have arisen by degradation of lactose and fragment recombination like other furans having more than six carbon atoms. Their flavor has been described by expert tasters as very similar to that of 2,4-decadienal, a known product of oxidized milk fat, and can be detected in skim milk below 0.1 ppm. This finding is significant because the oxidized flavor in dried milk products has been invariably associated with aliphatic aldehydes of lipid origin. Because of the flavor properties of the above heterocyclic aldehydes, the carbohydrates also must be possible precursors of the oxidized flavor in dairy products. If the presence of 5-(2'-furfuryl)-2-furaldehyde and 5-(5'-methyl-2'-furfuryl)-2-furaldehyde, or of any other carbohydrate-derived aldehyde having similar properties, can be demonstrated in skim milk powder or in sterile concentrated milk, measures to control the development of the oxidized flavor will have to include means of eliminating or retarding the Maillard reaction.

In conclusion, lactose-casein and whey browning systems in the dry state yield two sets of volatile compounds which are similar but not identical, the main differences being the absence of alkylpyrazines in heated whey, and the relatively large amount of  $\beta$ -hydroxy- $\gamma$ -butyrolactone in lactose-casein.

### References

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